

A bird-like vocalization in a treefrog (Anura, Rhacophoridae): Analysis of advertisement call characteristics in *Gracixalus weii*

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Abstract

The genus *Gracixalus* is a group of small and medium-sized rhacophorid frogs for which many new species have been described recently, yet little is known about their biology. *Gracixalus weii* is a species recently described in Guizhou Province, China, whose acoustic characterization was lacking. The advertisement call has a central role in the reproduction of frogs. We present the first detailed acoustic description for *G. weii* based on recordings from six individuals obtained at the type locality, Leigongshan Nature Reserve. We collected and analyzed 182 calls, and classified them into two different types (Type A and Type B) based on their call structure and note composition. Type A calls (33 calls from two males) consist of an introductory note followed by two click notes, with a mean total duration of 726.59 ± 119.11 ms and a dominant frequency of 2.46 ± 0.10 kHz. Type B calls (149 calls from all six males) comprise an introductory note and a single click note, mean 481.07 ± 77.03 ms in call duration, with a mean dominant frequency of 2.58 ± 0.15 kHz. Both call types exhibit broad frequency ranges (2.0–21.0 kHz). Among individuals possessing both type A and type B calls, type A calls account for 41.9%–69.0% of the vocalizations, while type B calls comprise 31.0%–58.1%. We found that the type A advertisement call of *G. weii* is similar to the song structure of the thrush *Turdus dissimilis*. The complex spectral structure may suggest a case of evolutionary convergence between certain anurans and birds in the use of acoustic signals. The advertisement calls of *G. weii* are notably distinct from those of other known *Gracixalus* species. These differences provide acoustic evidence supporting the species taxonomic validity. Our findings underscore the value of bioacoustic data in species identification and contribute basic information for future study on the behavior, ecology, and diversity of the understudied *Gracixalus* genus.

Key Words

bioacoustics, bird-like sound, broad frequency range, frogs, southwest China

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Introduction

The genus *Gracixalus* Delorme, Dubois, Grosjean & Ohler, 2005, a group comprised of small to medium-sized rhacophorids (Boruah et al. 2023), was erected by Delorme et al. (2005) and currently comprises 23 species (Frost 2025). Geographically, the *Gracixalus* genus is dispersed from Myanmar and western Thailand to Laos, central and northern Vietnam, and further to southern and southwestern China (Boruah et al. 2023; Tran et al. 2023). More than 50% of *Gracixalus* species have been described in recent years, indicating that the species diversity of *Gracixalus* has been poorly understood (Frost 2025). Furthermore, recent phylogenetic studies conducted by Matsui et al. (2017) and Chen et al. (2018) have identified several unnamed, distinct lineages within the group of *G. jinxiuensis*. These findings suggest that the species richness of *Gracixalus* is still underestimated. Despite this considerable number of discoveries and publications, the vocalizations of many *Gracixalus* species remain unknown (Boruah et al. 2023). To solve the above problems, morphological, genetic, and bioacoustic evidence should be provided to reveal the species diversity within the genus *Gracixalus*.

In anurans, male advertisement calls function as acoustic signals that affect mate choice and reproductive isolation, thereby shaping evolutionary diversification (Kelley 2004; Chen et al. 2024). Advertisement calls are species-specific courtship signals (Köhler et al. 2017) widely used in systematics, sexual selection, and call evolution studies (Zhu et al. 2021; Shen et al. 2024). Anuran vocal signals are shaped by phylogenetic history, ecological context, and the morphology of the vocal apparatus (Morton 1975; Ryan et al. 1988; McLean et al. 2013). Bioacoustic data remain scarce for most *Gracixalus* species, despite recent taxonomic revisions. Currently, among the 23 *Gracixalus* species identified worldwide, advertisement calls have been described for 10 species. Most descriptions are based on vocalizations from single individuals, which limits deeper understanding of intra-specific variation (Wang et al. 2018; Wu et al. 2025).

Gracixalus weii Liu, Peng, Wang, Feng, Shen, Li, Chen, Su & Tang, 2025 (Anura, Rhacophoridae) is a newly described species in southwest China (Liu et al. 2025). *Gracixalus weii* was originally described as the sister taxon of *Gracixalus jinxiuensis* Hu, 1978 based on 16S mtDNA and morphological traits, but notably was not compared to *Gracixalus huaping* Luo, Zhang, Pan & Yu, 2025, described in the same issue of the same journal (Luo et al. 2025). The potential phylogenetic proximity among these species warrants comparative acoustic investigation. Here, we present the first detailed acoustic description for this species. In addition, we found that the vocalizations of *G. weii* are similar to those of birds, providing a new perspective for the convergent evolution of biological vocalizations. Finally, we compare the advertisement calls of species in the genus *Gracixalus*, and the results provide acoustic evidence that supports the taxonomic differentiation of *G. weii* and contribute basic data for future behavioral and ecological studies on *Gracixalus* species.

Materials and methods

Call recordings

A total of 182 calls were recorded from six individuals of *G. weii*, collected from its type locality Leigongshan Nature Reserve, Leishan County, Guizhou Province, China (26.39°N, 108.08°E, ca. 1570 m elev., 15.7–20.5 °C air temperature, 85–97% ambient humidity) on April 20–26, 2025, between 19:00–23:00 h. Previous studies have shown that temperature can affect the spectral and temporal characteristics of frog vocalizations, so we used a portable temperature and humidity meter (SMART SENSOR AS817 stated accuracy ± 0.5 °C) to record temperature and humidity data in real-time (Zweifel 1959, 1970; Heinzmann 1970). This species is commonly found in bamboo forests near streams. The habitat of *G. weii* is shared with *Boulenophrys leishanensis* Li, Xu, Liu, Jiang, Wei & Wang, 2018, *Boulenophrys spinata* Liu & Hu, 1973, *Leptobrachella wulingensis* Qian, Xia, Cao, Xiao & Yang, 2020, and *Leptobrachium leishanensis* Liu & Hu, 1973. Calls of each individual were obtained using a digital recorder, SONY ICD-PX470 (sampling rate 44.1 kHz, 16-bit resolution). The SONY ICD-PX470 recorder exhibits a non-linear frequency response across its full frequency range. Each call was recorded within a 1 m distance from the calling individual. Recorded calls were always of isolated individuals and never from a mixed chorus. The recordings were saved as WAV files. The recordings are publicly available in Figshare at <https://doi.org/10.6084/m9.figshare.29236706>.

Snout vent lengths (SVLs) of all recorded males were measured in situ using a precision digital calliper to the nearest 0.1 mm. One *G. weii* (specimen number: LS20250426001) was collected for species identification and the others were released to their original habitat after measurement. After taking photographs, it was euthanized using isoflurane and then the specimen was fixed in 10% buffered formalin. Muscle tissue from the thigh was collected and preserved in 95% ethanol before fixation. The specimen was deposited in the Leigongshan National Nature Reserve Administration, China. Mitochondrial 16S gene were extracted and amplified from muscle samples of all samples and sequenced, and the obtained sequence was verified and uploaded to China National Center for Bioinformation (NGDC, <https://ngdc.cncb.ac.cn/genbase/submit/new?lang=zh>). The sequencing results were compared to finally determine the species collected. For the morphological identification, the procedure described by Liu et al. (2025) was followed.

Acoustic analyses

The advertisement call characteristics were analyzed with the software Raven Pro 1.6 (K. Lisa Yang Center for Conservation Bioacoustics [at the Cornell Lab of Ornithology] 2023). Temporal properties were measured

using Raven’s waveform display. Spectral properties were measured by averaging the spectrum over the entire duration of a call (Hann window, FFT = 512 samples, overlap = 50%, Hop Size = 256 samples). Our study used an FFT size of 512 points with a 50% overlap, which can achieve a balance between time resolution and frequency resolution, resulting in smoother spectrograms. We applied a Hann window to reduce spectral leakage and normalized the amplitude of all calls by scaling them to a uniform range between -1 and 1. Only calls that had a high signal-to-noise ratio and were free from overlapping calls of nearby males were used for the analysis. We used “call-centered” terminology as summarized by Köhler et al. (2017), in which the fundamental unit was defined as a “call”, and the main subunit of a call was defined as a “note”. We measured all parameters and characteristics following the procedure described by Köhler et al. (2017) and Wang et al. (2018) including (1) call duration (ms), CD (2) call intervals, CI (3) call repetition rate (calls/min), CR (4) first note duration, FND (5) second note duration, SND (6) third note duration, TND (7) first note interval, FNI (8) second note interval, SNI (9) dominant frequency (kHz), DF (Table 1). Oscillograms and spectrograms were generated using Seewave v.2.2.0 (Sueur et al. 2008) and TuneR 1.4.2 (Ligges et al. 2018) packages in R programme 4.2.2 (R Core Team

2021) with a “Hanning” window size of 256 samples and an overlap of 50%. Descriptive statistics of call characteristics: mean, standard deviation (SD), and range were computed using SPSS 23.0. We used principal component analysis (PCA) to examine the intraspecific variation of advertisement calls in *G. weii*. For the human ear, the advertisement call of *G. weii* is very similar to the song of birds in the Passeriformes order. Therefore, we referred to the bird songs recorded in the “The CNG Field Guide to the Birds of China” (Liu and Chen 2021), and also consulted local ornithologists. We plot oscillograms and spectrograms of the relevant bird song. Furthermore, we compared the advertisement calls of *G. weii* and its closely related species.

Results

Males were observed calling on or within bamboo (Fig. 1A). The habitat of *G. weii* is surrounded by dense bamboo forests (Fig. 1B). After molecular identification, we determined that the species captured in the Leigongshan Nature Reserve is *G. weii* (Fig. 1C). The 16S of NGDC accession number is C_AA130857.1 (specimen number: LS20250426001). The SVL of *G. weii* ranged from 30.5 mm to 33.3 mm (Table 1).

Table 1. Descriptive statistics for call parameters of advertisement calls of *Gracixalus weii*. Mean ± SD (Range).

Voucher		LS20250426001	LS20250426002	LS20250426003	LS20250421001	LS20250420001	LS20250421002	All individuals
Temperature (°C)		15.7	15.7	15.7	20.5	19.2	20.5	15.7–20.5
Humidity (%)		97	97	97	85	93	85%	85–97
SVL (mm)		33.3	30.5	31.9	32.4	32.9	32.0	30.5–33.3
Type A	Calls analyzed	13	Not recorded	20	Not recorded	Not recorded	Not recorded	2/33
	Call duration (ms)	584 ± 23 547–628	/	819 ± 26 782–862	/	/	/	727 ± 119 547–862
	First note duration (ms)	287 ± 18 262–327	/	354 ± 19 327–386	/	/	/	328 ± 38 262–386
	Second note duration (ms)	44 ± 4 39–50	/	59 ± 6 49–75	/	/	/	53 ± 9 39–75
	Third note duration (ms)	44 ± 4 38–51	/	74 ± 13 54–92	/	/	/	62 ± 18 38–92
	First note interval (ms)	92 ± 4 85–101	/	165 ± 6 151–177	/	/	/	137 ± 37 85–177
	Second note interval (ms)	118 ± 5 108–125	/	166 ± 10 149–195	/	/	/	147 ± 26 108–195
	Dominant frequency (kHz)	2.41 ± 0.14 2.15–2.67	/	2.50	/	/	/	2.46 ± 0.10 2.15–2.67
Type B	Calls analyzed	18	50	9	25	28	19	6/149
	Call duration (ms)	450 ± 32 391–500	551 ± 22 492–590	636 ± 36 567–680	395 ± 13 374–423	412 ± 16 375–438	469 ± 29 388–517	481 ± 77 374–680
	Call interval (ms)	13514 ± 3762 5218–21973	10699 ± 3654 5779–19359	22752 ± 6115 15707–39054	10107 ± 1690 6990–12955	7172 ± 2906 1027–14477	16931 ± 5457 8372–29106	12891 ± 6282 1027–39054
	Call repetition rate (calls/min)	4.60	5.55	2.79	6.86	8.22	4.03	2.79–8.22
	First note duration (ms)	308 ± 30 249–352	308 ± 15 276–345	383 ± 34 315–430	262 ± 10 248–284	272 ± 15 237–299	299 ± 33 219–356	297 ± 36 219–430
	Second note duration (ms)	47 ± 3 40–50	101 ± 16 73–138	88 ± 19 63–125	51 ± 9; 34–71	60 ± 15 38–92	75 ± 14 47–98	74 ± 26 34–138
	Note interval (ms)	95 ± 5 87–108	142 ± 18 94–189	165 ± 11 145–182	83 ± 7 62–99	79 ± 8 62–90	95 ± 16 73–133	110 ± 32 62–189
	Dominant frequency (kHz)	2.34 ± 0.10 2.07–2.41	2.73 ± 0.05 2.67–2.84	2.50	2.64 ± 0.11 2.41–2.76	2.46 ± 0.04 2.41–2.50	2.53 ± 0.05 2.50–2.67	2.58 ± 0.15 2.07–2.84



Figure 1. Photograph of the specimen and habitat. **A.** Adult male of *Gracixalus weii* inhabits bamboo; **B.** Habitat of *Gracixalus weii* in the type locality, Leishan County, Guizhou Province, China; **C.** Adult male of *Gracixalus weii* (LS20250426001) from Leishan County, Guizhou Province, China.

We recorded and analyzed the spontaneous vocalizations of 182 calls from six *G. weii* males. Measurements of call parameters are shown in Table 1. The advertisement call of *G. weii* does not have obvious pulses, but there are many harmonics (Fig. 2). Based on parameters such as call duration, call structure and number of notes of the advertisement calls of *G. weii*, we divide its advertisement calls into two types (Table 1). The two types of advertisement calls are usually mixed together and emitted (Fig. 2A). The advertisement call of *G. weii* has an “introductory note” followed by one or two “click notes” (Fig. 2B, C).

We only found Type A calls in two individuals and collected a total of 33 Type A calls. An individual with specimen number LS20250426001 recorded 13 type A advertisement calls, accounting for 41.9% of its vocal repertoire. The individual with specimen number LS20250426003 emitted 20 type A advertisement calls, comprising 69.0% of its vocal repertoire (Table 1). Each Type A call consists of an introductory note followed by two uniform click notes (Fig. 2B), with a total call duration ranging from 547 to 862 ms (mean $\pm$ SD: 727 ± 119 ms, $N = 33$). The first note (introductory note) has a duration of 262–386 ms (mean $\pm$ SD: 328 ± 38 ms). The second note (the first click note) is shorter, with a duration of 39–75 ms (mean $\pm$ SD: 53 ± 9 ms). The third note (the second click note) is similar to the second,

with a duration of 38–92 ms (mean $\pm$ SD: 62 ± 18 ms). The interval between the first note (introductory note) and the second note (the first click note) ranges from 85 to 177 ms (mean $\pm$ SD: 137 ± 37 ms), while the interval between the second and third notes ranges from 108 to 195 ms (mean $\pm$ SD: 147 ± 26 ms). Type A calls contain multiple harmonics and cover a broad frequency range of 2.0–21.0 kHz, with a mean dominant frequency of 2.46 ± 0.10 kHz (Fig. 2B).

All individuals have type B advertisement calls. We collected a total of 149 type B calls. The number of recorded type B calls varied among individuals, ranging from a minimum of 9 to a maximum of 50 calls (Table 1). Type B call of *G. weii* is composed of an introductory note followed by a single click note (Fig. 2C), together being 374–680 ms call duration (mean $\pm$ SD: 481 ± 77 ms, $N = 149$). The mean call interval was 12891 ± 6282 ms, ranging from 1027–39054 ms. The call repetition rate ranged from 2.79–8.22 calls/min. The first note (introductory note) had a duration of 219–430 ms (mean $\pm$ SD: 297 ± 36 ms), and the second note (click note) duration was 34–138 ms (mean $\pm$ SD: 74 ± 26 ms). The note interval was 62–189 ms (mean $\pm$ SD: 110 ± 32 ms). The calls of type B and type A are similar and have a broad frequency range (Fig. 2C), type B calls with a mean dominant frequency of 2.58 ± 0.15 kHz.

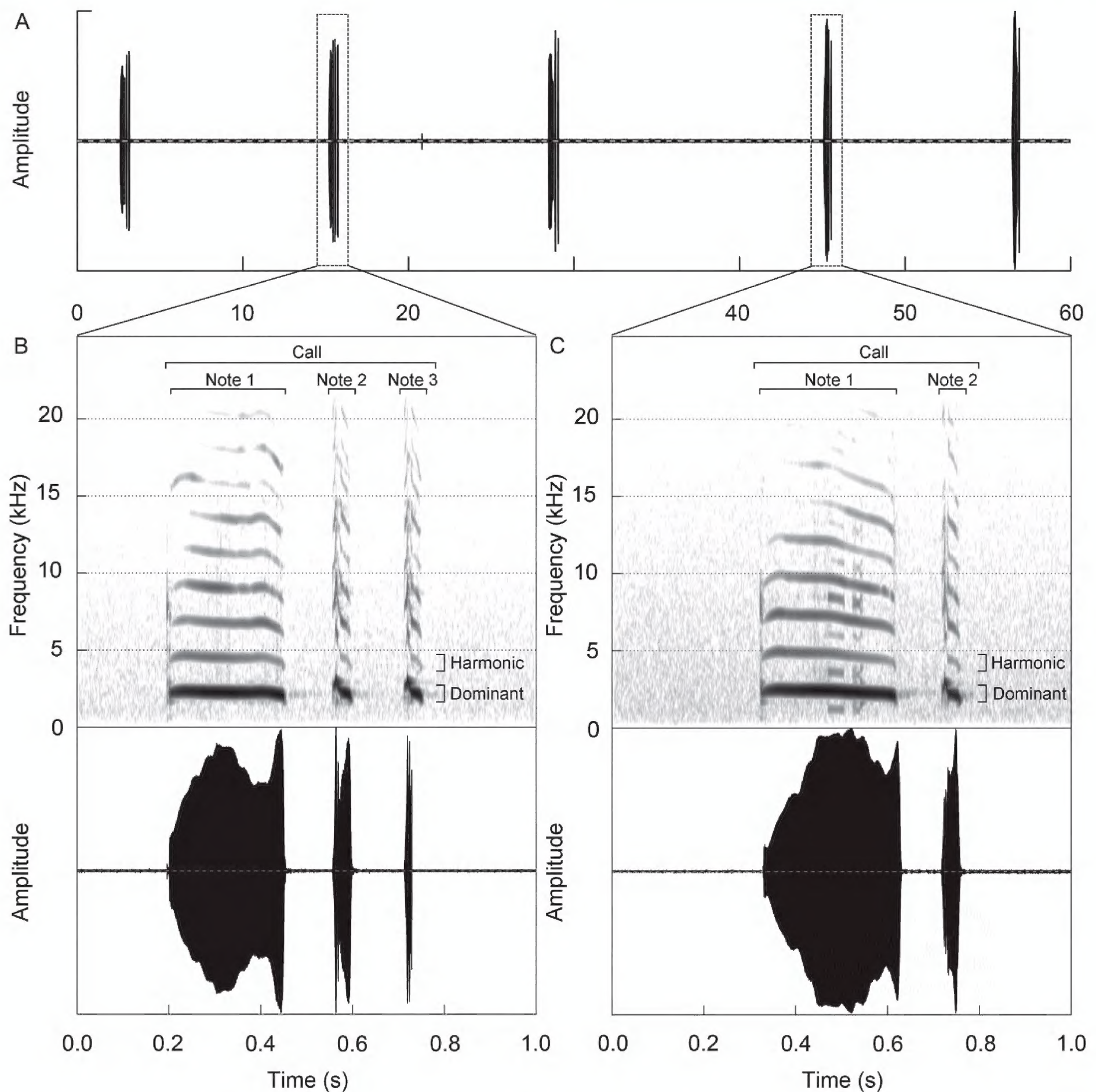


Figure 2. Advertisement calls of the *Gracixalus weii*. **A.** 60 s oscillograms showing five calls of *Gracixalus weii*; **B.** 1 s oscillograms and corresponding spectrograms showing one type A call of *G. weii*; **C.** 1 s oscillograms and corresponding spectrograms showing one type B call of *G. weii*.

In the principal component analysis (PCA) of individual variation in *G. weii*, the first two principal components explained 82.49% of the variation (Table 2). The PC1 axis accounted for 52.62% of the total variation and was primarily associated with temporal parameters, as it comprised the number of notes, third note duration, and call duration (Table 2). The PC2 axis explains 29.87% of the variation, primarily driven by the second note duration (Table 2). On the plot of PC1 vs. PC2, the type B advertisement calls of *G. weii* cluster together and exhibit limited inter-individual variation (Fig. 3). The type A and type B advertisement calls are clearly distinguishable from each other (Fig. 3).

Auditorily, the call of *G. weii* is similar to a bird-like chirp. We found that the advertisement call of *G. weii* is similar to the song of the Black-breasted Thrush *Turdus dissimilis* Blyth, 1847 (Fig. 4A). As is well known, the songs of birds are more melodious and varied than those of frogs. Note that the calls of *G. weii* we believe to be similar to those of *T. dissimilis* are not entirely identical. In order to better demonstrate the similarity of their vocalizations, we selected the segment with the highest similarity and plotted the oscillograms and corresponding spectrograms of *T. dissimilis* (Fig. 4B). At first hearing, the song structure of *T. dissimilis* is similar to the type A advertisement call of *G. weii*. Firstly, both of them produce a longer note

Table 2. Factor loadings of the Principal Component Analysis (PCA) on the advertisement call parameters of the *Gracixalus weii*.

Call parameters	Principal components		
	1	2	3
Call duration	0.915	0.389	-0.068
Dominant frequency	-0.233	0.697	0.597
Number of notes	0.940	-0.267	0.198
First note duration	0.586	0.472	-0.586
Second note duration	-0.137	0.882	0.058
Third note duration	0.940	-0.267	0.198
First note interval	0.550	0.733	-0.008
Second note interval	0.940	-0.267	0.198
Eigenvalue	4.210	2.390	0.825
Variance (%)	52.621	29.874	10.317
Cumulative variance (%)	52.621	82.495	92.813

followed by two shorter notes (Fig. 2B, 4B). In addition, the song duration of *T. dissimilis* was 726 ms, with a dominant frequency of 2.07 kHz, which is very similar to the type A call parameters of *G. weii* (Table 1).

We compared the advertisement calls of *Gracixalus* species (Table 3). The calls emitted by males of *G. weii* differ considerably from those previously described for other species of the genus *Gracixalus*, reinforcing the specific identity of the taxon (Table 2). For example, *G. weii* has more notes than those emitted by males of *G. huaping* (one note), *Gracixalus tianlinensis* (one note). The number of notes of *G. weii* is much lower than that of *Gracixalus nonggangensis* (40–72 notes). The duration of click notes of *G. weii* is longer than that of *Gracixalus gracilipes* (6–20 ms), *Gracixalus quangi* (10 ms), and *Gracixalus supercornutus* (6–9 ms). The frequency range of the advertisement call of *G. weii* is much larger than that of *Gracixalus guangdongensis*, *Gracixalus liusanjieae*, *Gracixalus jinggangensis* and *G. jinxiuensis*.

Discussion

Over the last decade, morphological and phylogenetic analyses have facilitated the identification of species in the genus *Gracixalus*, which have increased from nine to twenty-three (Liu et al. 2025; Luo et al. 2025; Wu et al. 2025).

Table 3. Comparative call parameters of *Gracixalus*.

Species	Temperature	Number of notes	Call duration (ms)	Duration of the introductory note (ms)	Duration of the click note (ms)	Dominant frequency (kHz)	Frequency range (kHz)	Reference
<i>G. weii</i>	15.7–20.5	2	374–680	219–430	34–138	2.07–2.84	2.0–21.0	This study
		3	547–862	262–386	194–319	2.15–2.67	2.0–21.0	This study
<i>G. gracilipes</i>	7.3–18.1	/	/	150–250	6–20	4.1–5.1	4.1–22.0	Rowley et al. 2015
<i>G. guangdongensis</i>	17–21	2	500–650	360–470	40–50	2.4– 4	2.0–3.0	Wang et al. 2018
<i>G. huaping</i>	20	1	610–690	/	/	2.89	2.10–3.37	Wu et al. 2025
<i>G. jinggangensis</i>	17.6	3–4	447–620	147–219	42–67	2.6	2.0–3.1	Zeng et al. 2017
<i>G. jinxiuensis</i>	22	2	460–610	/	/	2.50	2.08–2.95	Wu et al. 2025
<i>G. liusanjieae</i>	15	2	660–800	/	/	2.58	2.25–2.95	Wu et al. 2025
<i>G. nonggangensis</i>	17	40–72	15060	490	120	2.2	1.6–2.7	Wang et al. 2018
<i>G. quangi</i>	24.1	/	/	370	10	4.1–4.7	4.1–18.8	Rowley et al. 2011
<i>G. supercornutus</i>	22.6–23.7	/	/	370	6–9	3.6–4.1	3.6–20.1	Rowley et al. 2015
<i>G. tianlinensis</i>	18	1	/	/	/	2–3	2.0–5.5	Chen et al. 2018

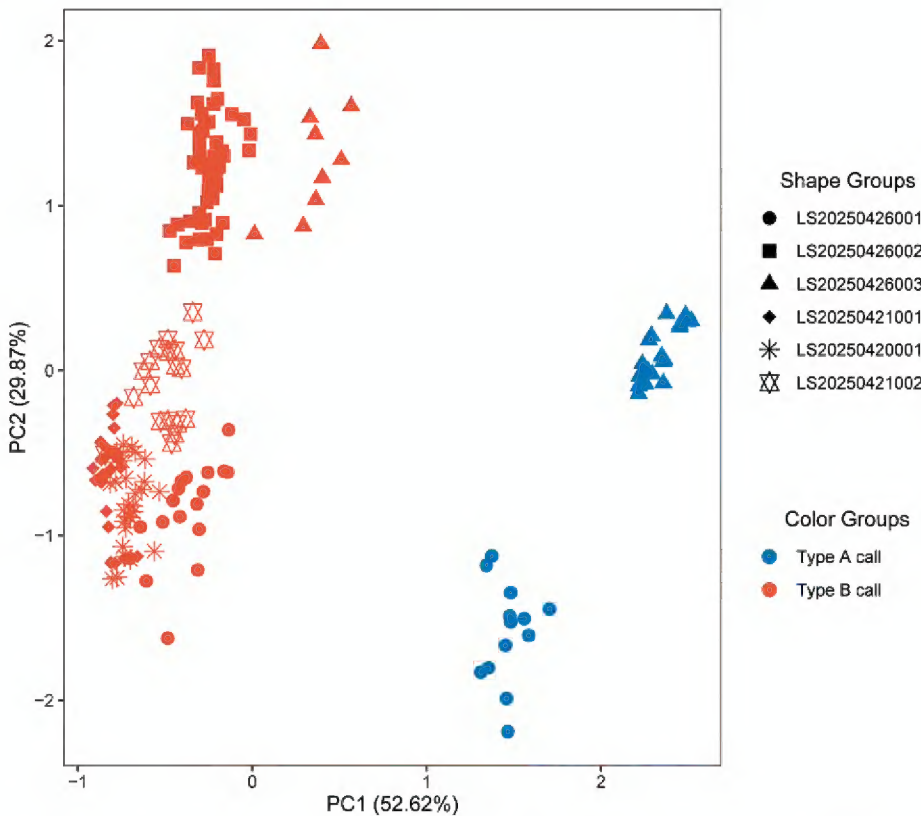


Figure 3. Plots of the first principal component (PC1) versus the second (PC2) for six individuals of advertisement calls of *Gracixalus weii* from a principal component analysis. Six different symbols represent six individuals, and two colors represent two types of advertisement calls for *G. weii*.

Gracixalus weii is a newly described species with the type locality in Leigongshan Nature Reserve, Guizhou Province. In fact, more than ten new amphibian species have been discovered and described in Guizhou Province in recent years (Li et al. 2024; Amphibia China 2025). It is worth noting that *G. weii* is the only species of the *Gracixalus* genus discovered in Guizhou Province so far (Liu et al. 2025). This further highlights the uniqueness of *G. weii* and suggests that the diversity of amphibians in Guizhou Province remains underestimated.

Bioacoustics, behavioral and natural history information for species of the genus *Gracixalus* is very scarce. Among cryptic species, the use of acoustic diagnostic features for identification could be an alternative to morphometric and molecular diagnosis (Rivera-Correa et al. 2022; Batallas and Brito 2023). The advertisement calls of *G. weii* have different characteristics from other species in the *Gracixalus* genus, which supports their specificity in acoustic signals at the species level. However, the magnitude of cryptic diversity and their advertisement calls remain largely unknown (Rowley et al. 2015; Zeng et al. 2017).

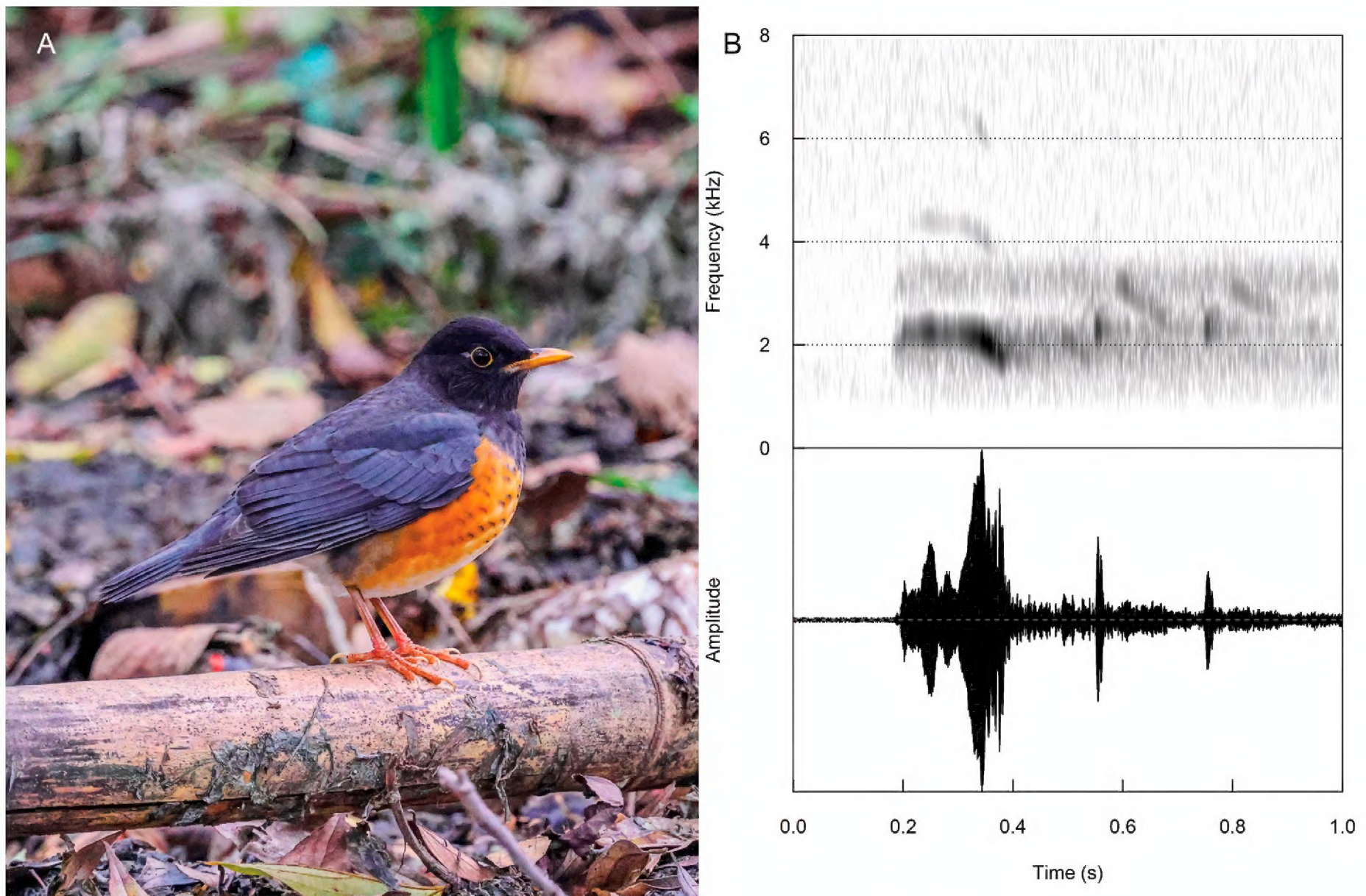


Figure 4. Song of *Turdus dissimilis*. **A.** Photograph of *T. dissimilis*. **B.** 1 s oscillograms and corresponding spectrograms showing song of *T. dissimilis*.

In the wild, *Gracixalus* usually hide inside bamboo (Fig. 1A; Amphibia China 2025), indicating that it is difficult to record *Gracixalus* through direct visual contact. In addition, the advertisement call of *G. weii* is very similar to the song of birds, which can easily lead to misjudgment and underestimation of the amphibian diversity in the survey area during field investigations. Therefore, in this study, we provided a detailed description of the advertisement call characteristics of the *G. weii*.

The bird-like tonal structure likely represents a case of acoustic convergence with avian communication systems under similar ecological constraints. This phenomenon is consistent with the acoustic convergence recorded by Dubois and Martens (1984) in the Himalayan rapids between the genus *Nanorana* Günther, 1896 of frogs and the bird *Phylloscopus maginostrostris* Blyth, 1843. This convergence suggests that the evolution of acoustic signals in amphibians is not only influenced by intraspecific identification and sexual selection, but may also be influenced by broader ecological interactions, including heterogeneous signal environments (Morton 1975). Future experimental work, especially through replay experiments to study the response of *G. weii* and *T. dissimilis* to each other's calls, is crucial for testing the functional significance of this acoustic convergence phenomenon.

The advertisement call of *G. weii* consists of variable non-stereotype and non-pulsatile call types, ranging from a relatively longer introductory note to short click notes with broad frequency range (Fig. 2). The structure of introductory notes and click notes in each call is roughly

similar. The evolution of advertisement call was influenced by both historical factors (phylogeny) and external factors (calling sites) (Bosch and De la Riva 2004; McLean et al. 2013; Escalona et al. 2018). From a phylogenetic perspective, *G. weii* is nested within the *G. jinggangensis* species group (Liu et al. 2025). However, the frequency range of *G. jinggangensis*, *G. liusanjieae*, *G. jinxiuensis*, and *G. huaping* are significantly narrower than that of *G. weii* (Zeng et al. 2017; Wu et al. 2025). Other species of *Gracixalus* with a broad frequency range, such as *G. gracilipes*, *G. quangi*, and *G. supercornutus*, show greater genetic distance from *G. weii* (Rowley et al. 2011, 2015; Liu et al. 2025). Therefore, comparative data from phylogenetically close species like *G. huaping* and *G. jinxiuensis* would be particularly informative to determine whether this high-frequency, broadband pattern represents a derived or ancestral trait within the genus. Given its close phylogenetic affinity to *G. huaping* and *G. jinxiuensis*, *G. weii* may represent an intermediate stage in the diversification of acoustic strategies within *Gracixalus*.

Traditionally, frog vocalizations have been considered highly stereotypical, particularly in their role in promoting species recognition (Shen et al. 2023). However, recent studies have revealed that species of Rhacophoridae possess a complex vocal repertoire comprising distinct call types, which sometimes even appear in combinations of different vocalizations, such as *Kurixalus hainanus* (Liu et al. 2025). Our findings support this view as well. The advertisement calls of *G. weii* indicate that some individuals possess relatively complex acoustic signals.

We hypothesize that the unusually broad frequency range in *G. weii* may reflect adaptations to specific acoustic environments or unique morphological characteristics of its vocal organ. This strategy may help improve signal detection probability in stream habitats with complex environmental noise (Feng et al. 2006). Previous studies have suggested that species inhabiting grassland areas (i.e., regions with low woody vegetation density) are expected to produce acoustic signals characterized by shorter duration, higher frequency modulation (FM), higher peak frequencies, and broader frequency bandwidth, as these features are believed to reduce call attenuation and distortion (Erdtmann and Lima 2013; da Rosa et al. 2023). In contrast, *G. weii*, which inhabits dense bamboo forests with high vegetation density, exhibits an exceptionally broad frequency range. This may be due to more complex spectral structures or may evolve through sexual selection mechanisms (Shen et al. 2008). This could indicate rapid acoustic divergence, perhaps driven by sexual selection, similar to the “hyperextended vocal repertoires” reported in other Asian frogs (Rowley et al. 2015). In previous studies, female frogs may prefer more complex calls, possibly because such calls correlate with larger body size (Zhu et al. 2017; Liu et al. 2018) or better physical condition. Similarly, short click notes may play a role in male domain defense (Rowley et al. 2015). However, the confirmation of these behavioral functions still requires direct behavioral observation in the future for verification. It is noteworthy that future studies could employ analytical techniques with higher temporal resolution (such as shorter FFT windows or wavelet transforms) to precisely resolve the temporal-spectral microstructure of the short “click notes” within the calls and explore their potential biological functions.

In order to verify the preliminary hypothesis regarding the vocalization of *G. weii*, future research should focus on controlled behavior experiments. We propose: (1) playback experiments presenting different call types (A and B) of *G. weii*, heterospecific vocalizations of related species, and songs of *T. dissimilis* to male *G. weii* in natural or semi-natural settings to observe behavioral responses (e.g., vocalizations, approach, aggression) and distinguish between advertisement and territorial functions; and (2) phonotaxis experiments testing female preference for different call types to directly evaluate sexual selection hypotheses. Future work should also integrate more comprehensive acoustic, morphological, environmental, and genetic datasets, particularly using 3D microtomography and comparative laryngeal anatomy to test structural adaptations for broadband signal production. A comparative acoustic survey of these closely related species would provide valuable insights into signal evolution within the Rhacophoridae.

In summary, our study provides an initial description of the advertisement call characteristics of *G. weii* and supplies acoustic evidence to support its taxonomic identification. Our results offer basic data for future study in behavior, ecology, and biodiversity.

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